PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Docket No.: MORENO-LOPEZ

In re PATENT Application of:	)
	)
SONIA MORENO-LOPEZ &	) **Examiner: Anne Marie**
MARCOS TIMÓN-JIMENEZ	) **Sabrina Wehbe**
	)
Appl. No.: 10/816,465	) **Group Art Unit: 1633**
	)
Filed: April 1, 2004	) **Confirmation No.: 8524**
	)
For: MEANS FOR ELICITING AN IMMUNE RESPONSE AND A METHOD THEREFOR	)

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

CONSIDERED: /AW/ (03/30/2009)

DECLARATION UNDER RULE 131 (a)

S I R:

Sonia Moreno-Lopez and Marcos Timon-Jimenez depose and declare as follows:

1. We are co-inventors in the above identified patent application, which entered the USA national phase of PCTDE02/03798 on April 1, 2004 claiming priority from German patent applications Nos.: 101 48 697.9 of October 2, 2001 and 101 56678.6 of November 12, 2001.

2. In an Office Action dated July 23, 2008, the Examiner in the above application rejected claim 43 as anticipated by the publication by Schirmbeck et al., J. Mol Med (2001) 79: 343-350 and published May 3, 2001 (hereinafter: "Schirmbeck reference"). Claim 42 was deemed obvious over the Schirmbeck

reference in view of a publication by Makkerh et al. (1996) Current Biology, Vol. 6(8), 1025-1027.

3. We make this declaration to establish that we conceived and actually reduced to practice the invention before the date of publication of the Schirmbeck reference as supported by the material attached hereto as Exhibits A-E.

4. Attached hereto as Exhibit A are 4 pages, of which page 1 is a protocol on the coupling of the hairpin-shaped oligonucleotide, which subsequently close the ends of the covalent MIDGE-vector with the NLS peptide. The product is designated as NLS-MOL-GGGA-NH and later ligated with the MIDGE-vector. The cross linker provided is designated as sKMUS (referred to also on page 11, line 16 in the English translation of the WO 03/031469). This page bears a date of September 18, 2000. Page 2, titled Purification... is dated October 2, 2000. Page 3 which shows the agarose control gel with the obtained NLS-MOL-GGGA-NH- fractions as referred to on page 2. Page 4 shows another control gel with MOL-GGGA-NH-, NLS-MOL-GGA-NH-and TAT-MOL-GGA-NH fractions, dated October 2, 2000. Translations provided for pages 1 and 2 are behind the pages.

5. Attached hereto as Exhibit B are 11 pages showing the dates of the HPLC runs for purifying the oligonucleotide NLS-MOL-GGGA-NH, whose synthesis is shown on pages 1-4 in Exhibit A, and was carried out on October 2, 2000,.

6. Attached hereto as Exhibit C, are two excerpts from the lab book dated October 4, 2000 showing electrophoresis gels with the fractions of the NLS-MOL-GGGA-NH oligonucleotide successfully purified with HPLC. These are hairpin-shaped oligonucleotides from the HPLC runs from the 11 pages of Exhibit B which form part of the inventive MIDGE-constructs.

7. Attached hereto as Exhibit D is a page from a production protocol dated December 22, 2000 with a translation, which evidences the synthesis of the inventive MIDGE-NLS vector (here: MOK-HBsAGSAY1-NLS-M). from the prior

synthesized and HPLC purified NLS-MOL-GGGA-NH hairpin-shaped oligonucleotides and the MOL-D-oligonucleotide. A comparable MIDGE HbsAG NLS-construct is disclosed on pages 12-13 paragraph 4 of the English translation of WO 03/031469.

8. Attached hereto as Exhibit E is a page showing the data of the production of the NLS-coupled MIDGE vectors in association with the crosslinker sKMUS. Data as entered on December 19, 2000 remain in that data base.

9. The Exhibits A-E evidence that at all relevant times, we were in possession of the MIDGE-NLS prior to the date of the Schimbeck reference and that we were diligent in filing the patent applications.

The declarants further state that the above statements were made with the knowledge that willful false statements and the like are punishable by fine and/or imprisonment, or both under Section 1001 of Title 18 of Unites States Code, and that such willful false statements may jeopardize the validity of this application or any patent resulting therefrom.

Date_____

SONIA MORENO-LOPEZ

Date January 22nd 2009

MARCOS TIMÓN-JIMENEZ

EXHIBIT A

Produktname: NLS-MOL-666ANH Chargennummer: NLS-180900

Datum: 18.09.00

Oligonukleotid-Kopplung mit: [redacted]

Größe des Reaktionsansatzes: [redacted] µg Oligos

	Name	Chargen-Nr.	Konz. (µg/µl) o. mM	
Oligos:	[redacted]	[redacted]	6,5	= 1mM
zu koppelndes				
Molekül:	**NLS**		2 mM	
Crosslinker:	[redacted]	BE44012	50 mM	
MW (g/mol)				

Bemerkung: Peptid und Crosslinker-Lösungen vor der Reaktion frisch ansetz
in DMF 2083 µl 50mg

Reaktion der Oligos mit Crosslinker:

Volumen (µl)	Substanz	Konz.	einzuwiegende Masse an Crosslinker in mg
100	MOL-GGGA-NH	6,5 µg/µl	
100	sKMUS	50 mM	2,40
200	coupling buffer(pH 7.(	5x	
600	Wasser (Milipore)		

Inkubation: 120 min bei 37°C 1650 – 19°° → -20°C üN
~~(4x je 25 µl sKMUS nach 0,30,60 und 90 min zugeben)~~ 19.09.

Stoppen der Reaktion mit: 50 µl 1 M Tris HCl pH 7,5

1ml-Ansätze auf 3 Eppis verteilt: +33µl 3M NaAc, + 832,5µl EtOH p.a.
EtOH-Fällung: +6,6µl 1M $MgCl_2$ } 70% EtOH je 500µl

Volumen (µl)	Substanz	
100	3 M Natriumacetat	(=10 % des Ansatzes)
20	1 M MgCl2	(= 2% des Ansatzes)
2500	Ethanol (p.a.)	(= 2,5x Vol. des Ansatzes)

Inkubation: 30 min bei -70 °C; 30 min 4 °C u. 12.000 rpm zentrifugieren anschließend mit 1-2 ml EtOH (70 %)waschen, 15 min 4 °C u. 12.000 rpm zentrifugieren.

Kopplung mit NLS

	Volumen (µl)	
		je 270µl in 3 Ansätze, die 3 dann poole.
Pellet in	(800)	Wasser aufnehmen, auflösen lassen.
Dann	100	5x couplin buffer (pH 7.0)
	100	0 (2 mM bei Peptiden) zugeben.
Inkubation:	60 min bei 37 °C	

Product Name: NLS-Mol-GGGA-NH Batch No.: NLS - 180 900

Date: 09/18/00

Oligonucleotide coupling partner: NLS

Size of reaction batch: 650 **µg** Oligo Primer

	Name	Batch No.	Concentration (µg/µL) or mM	
Oligo Primer	**MOL-GGGA-NH**	180053	6.5	= 1 mM
Molecule				
to be coupled	**NLS**		2 mM	
Cross-linker	**sKMUS**	BE 44012	50 mM	
Mol. Weight (g/mol)	**480,47**			

Remark: Prepare peptide and cross-linker solution directly before start of reaction

→ in DMF 2083µl 50mg

Reaction of Oligo Primer with Cross-linker:

Volume (µL)	Substance	Concentration	Amount of Cross-linker in mg
100	MOL-GGGA-NH	6.5 µg/µL	2.40
100	sKMUS	50 mM	
200	coupling buffer (pH 7.6)	5x	
600	Water (Millipore)		

Incubation: 120 min. at 37°C 4:50 p.m. - 7:00 p.m. → -20°C overnight
~~(add 4x 25 µL sKMUS, after 0, 30, 60 and 90 min.)~~ 09/19 ?

Reaction termination accomplished by adding: 50 µL 1 M Tris HCL pH 7.5

1 mL batches, apportioned onto 3 reaction tubes: + 33µl 3M natrium acetate
+ 6.6 µl $MgCl_2$
+ 832,5 µl Ethanol (p.a.) (900µl of 70% Ethanol)

Ethanol precipitation:

Volume (µL)	Substance
100	3 M natrium acetate (= 10 % of reaction batch)
20	1 M $MgCl_2$ (= 2 % of reaction batch)
2500	Ethanol (per analysis) (= 2.5 x Volume of reaction batch)

Incubation: 30 min. at -70°C; 30 min. at 4°C and centrifugation at 12.000 rpm, subsequently washing with 1-2 mL Ethanol (70%), 15 min. at 4°C and centrifugation at 12.000 rpm.

Coupling with NLS

	Volume (µL)	
	→ 270 mL for each 1/3 reaction batch, then pool the 3 batches	
Dissolve pellet in	(800)	
then add	100	5 x coupling buffer (pH 7.0)
	100	0 (2 mM in case of peptides)
Incubation:	60 min. at 37°C	

Produktname: Chargennummer:

Datum:

Reinigung der Peptid gekoppelten Oligonukleotide mittels HPLC:

Laufsystem: ☒ 100 mM Ammoniumcarbonat / Acetonitril
O Wasser / Acetonitril

Gradient: von 0 % Acetonitril auf 30 % Acetonitril in 50 min

Fraktionen eindampfen und in Wasser aufnehmen.

Kontrolle der gesammelten HPLC-Fraktionen:

O 20 % PAGE-Gel
☒ 4 % Agarose-Gel

Peptid-Oligonukleotid-Fraktion rein ? ☒ **ja**
O **nein**

Wenn: **ja**
Dann: Freigabe der Peptid-Oligonukleotide.

Wenn: **nein**
Dann: erneute HPLC-Reinigung und Fällung der Fraktionen,
Kontrolle auf Agarose-Gel.

Datum/Unterschrift: 02.10.00 F. Sch

Product Name: Batch No.:

Date:

Purification of peptide-coupled oligonucleotides by HPLC

Mobile phase: ☒ 100 mM ammonium carbonate / acetonitrile

○ water / acetonitrile

Gradient: from 0% acetonitrile to 30% acetonitrile in 50 min.

Fractions have to be evaporated and re-dissolved in water.

Analysis of collected HPLC fractions:

○ 20% PAGE gel

☒ 4% agarose gel

Peptide-oligonucleotide fraction included? ☒ yes

○ no

If: **yes**

Then: approval of peptide-oligonucleotides

If: **no**

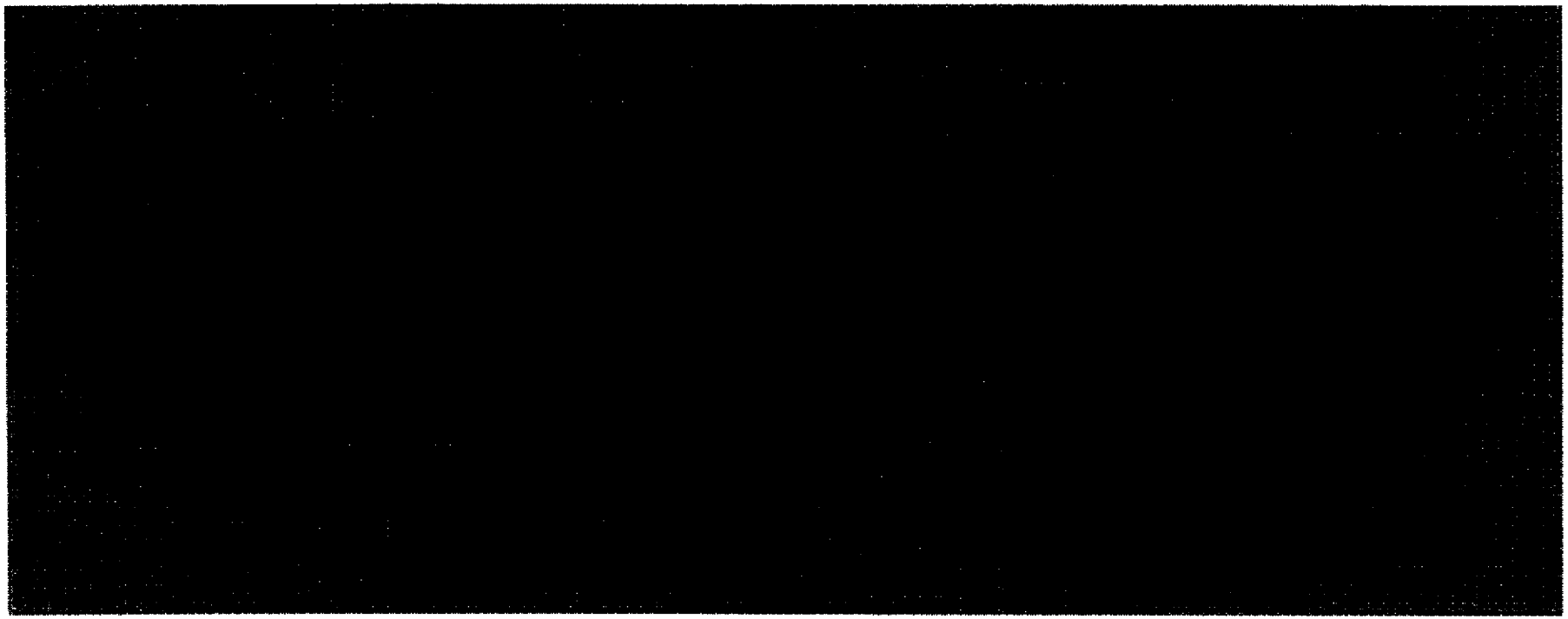
Then: a further HPLC purification and precipitation of fractions, analysis with agarose gel

Date / signature: 10/02/00

NLS-180900

NLSTATHPLCFraktionen

NLS-NOL666ANH



sidst and LB 6 S. ~~84-85~~ 82-83 FS

#124 & #125, MOL-GGGA-NLS & -TAT, mel 2000-10-26

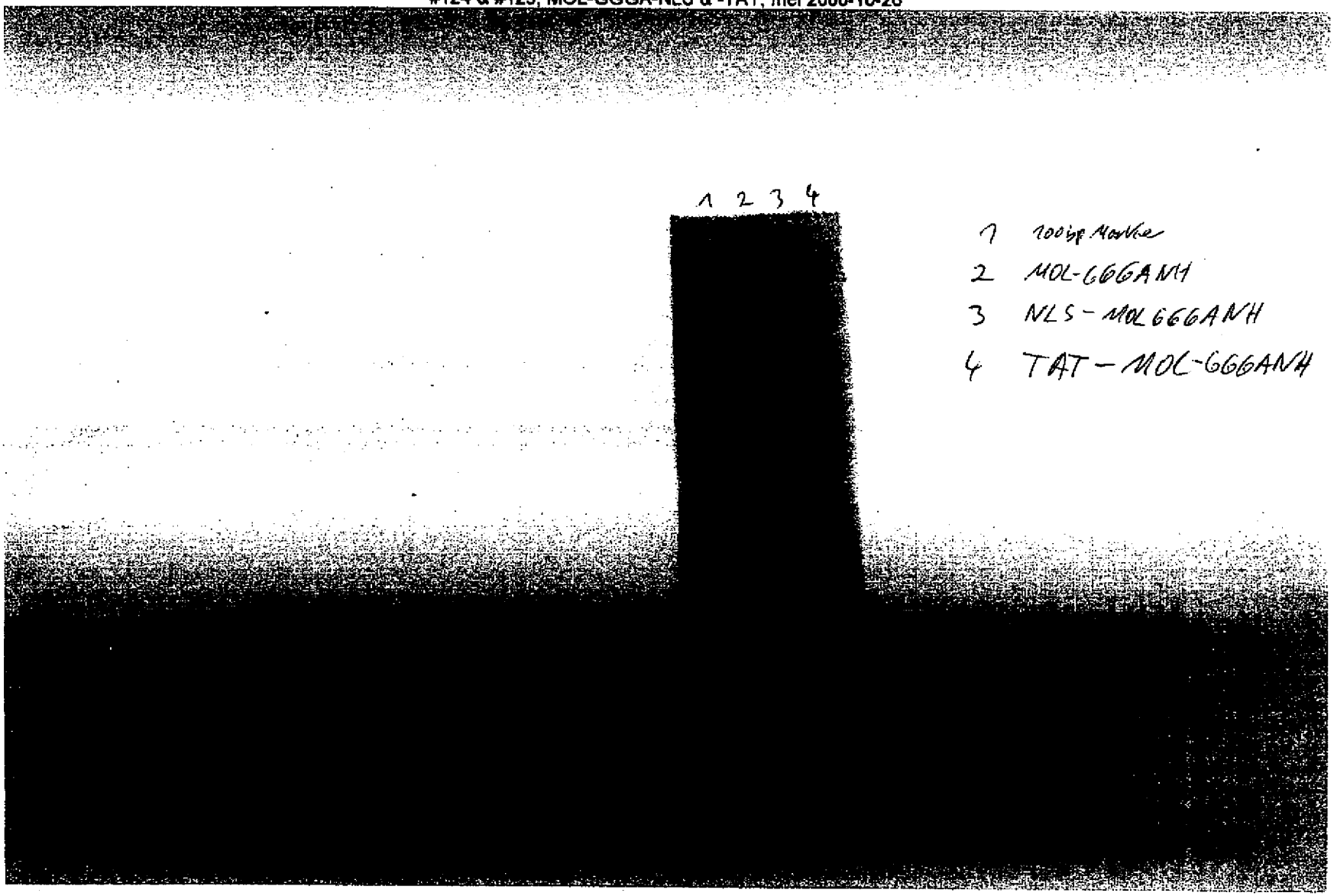


EXHIBIT B

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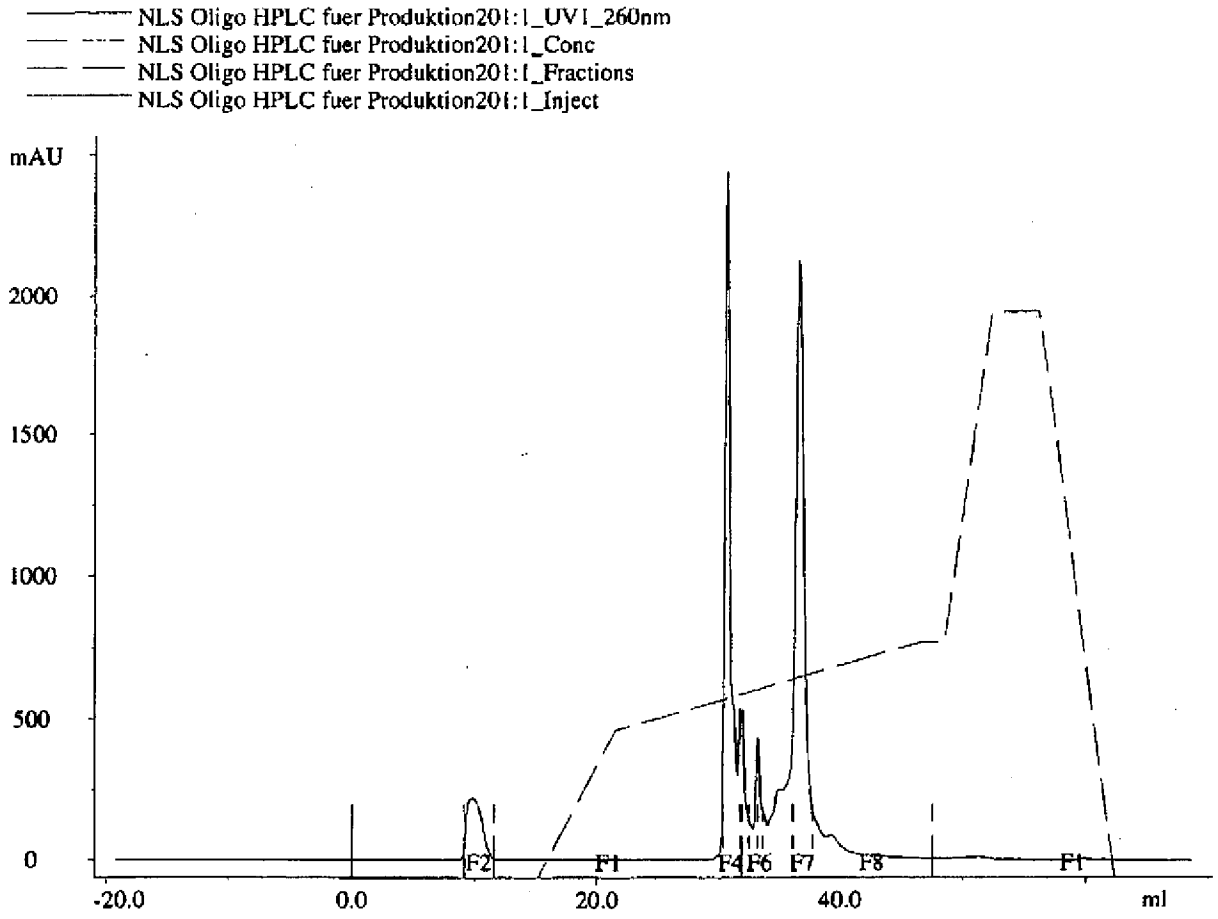
Method file: c:\...\Florian\NLS Oligo HPLC fuer Produktion2



Method Information

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Date of creation: 02.10.2000 17:05:09
Target system: System 1
Method last modified by: Florian
Date of last modification: 02.10.2000 17:05:09
Strategy name: B100_100
Strategy date: 01.08.2000 14:19:12
Strategy size: 620912 bytes

UNICORN V3.21
Result file: c:\...\Florian\NLS180900\NLS Oligo HPLC fuer Produktion201
Method file: c:\...\Florian\NLS Oligo HPLC fuer Produktion2

Variables

Saeule	Nucleosil_300_C18_250x8	
Flussrate	2.40	{ml/min}
Wavelength_1	260	{nm}
Wavelength_2	280	{nm}
Wavelength_3	215	{nm}
Pressure_limit	10.00	{MPa}
Equilibrate_with	1.50	{CV}
Empty_Loop_with	2.30	{ml}
Wash_column_with	1	{CV}
Target_ConcB_1	22.00	{%B}
Length_of_gradient_1	0.50	{base}
Target_ConcB_2	35	{%B}
Length_of_gradient_2	2.00	{base}
Conc_of_eluent_B	84	{%B}
Clean_with	0.60	{CV}
Reequilibrate_with	1.00	{CV}

Questions

No 1: Probenname:
NLS-MOL-GGGA-NH
No 2: Chargen-Nr. der NLS-Oligos
NLS-180900
No 3: Probenmenge:
1,5 ml
No 4: Säule:
Nucleosil-300 250x8mm
No 5: Batch-No. der Säule:
Knauer 2101s
No 6: Puffer A:
100 mM Ammoniumcarbonat
No 7: Puffer B:
80 % Acetonitril (+100mM Ammoniumcarbonat)
No 8: Bemerkungen:

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Run by: Florian 02.10.2000 18:10:37
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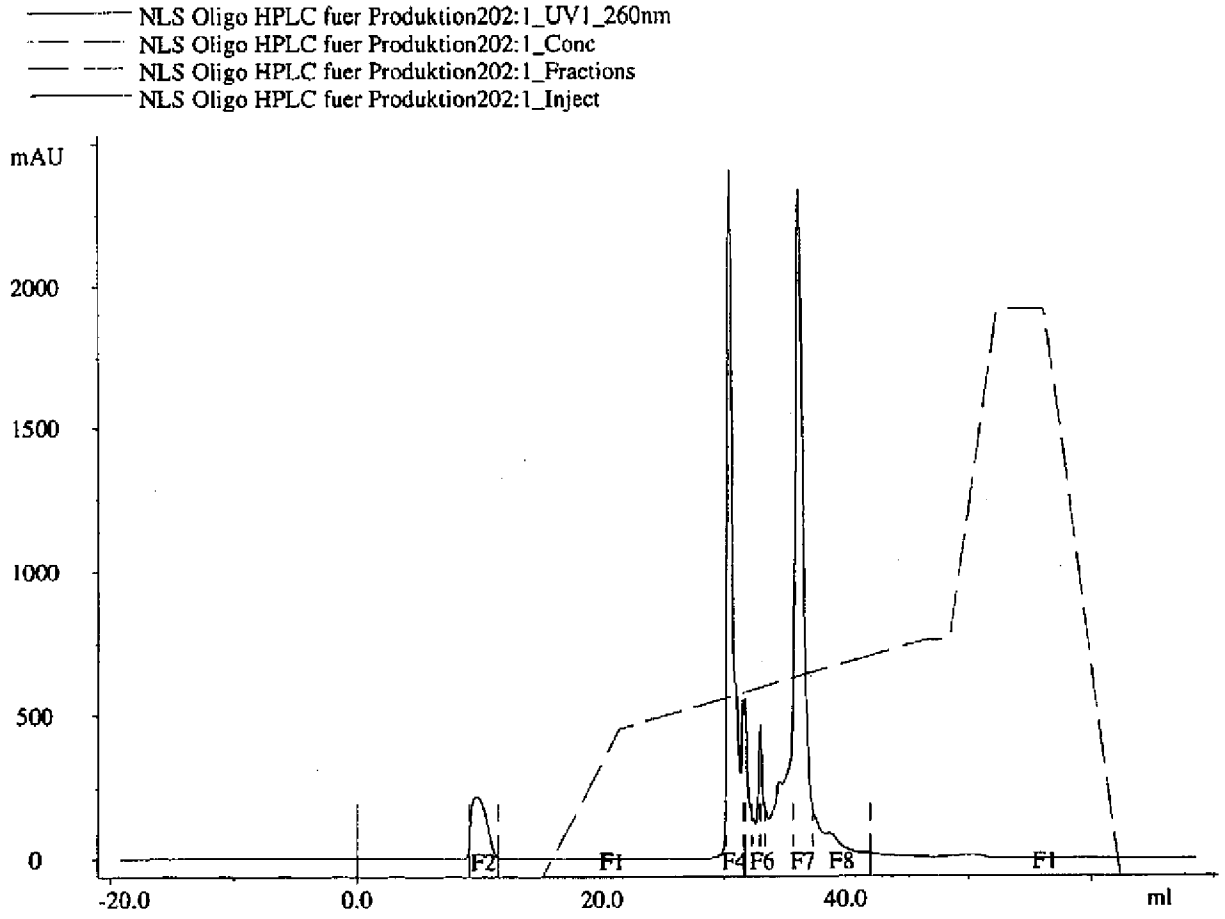
Method file: c:\...\Florian\NLS Oligo HPLC fuer Produktion2



Method Information

Method name: NLS Oligo HPLC fuer Produktion202
Method created by: Florian
Date of creation: 02.10.2000 17:59:23
Target system: System 1
Method last modified by: Florian
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Strategy name: B100_100
Strategy date: 01.08.2000 14:19:12
Strategy size: 620912 bytes

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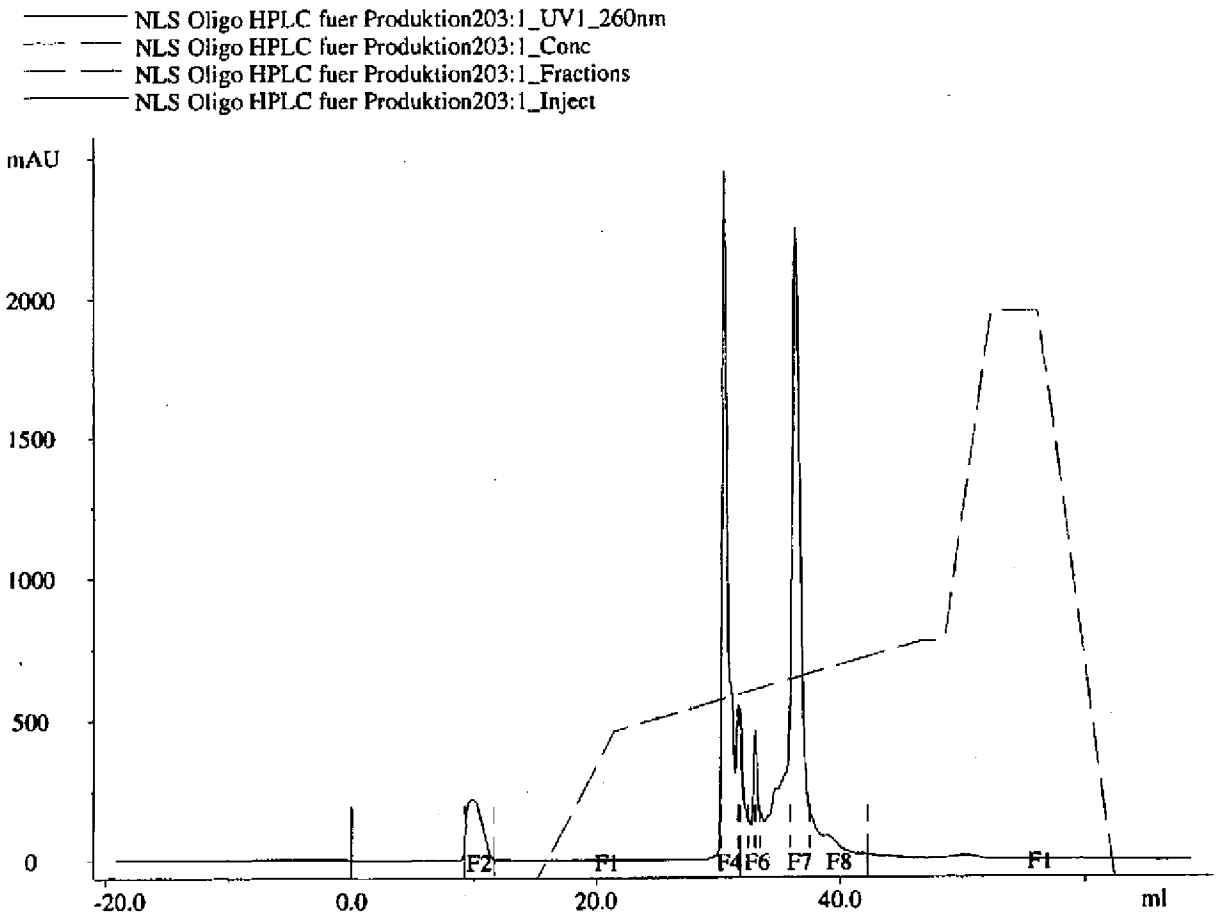
Method file: c:\...\Florian\NLS Oligo HPLC fuer Produktion2



Method Information

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Date of creation: 02.10.2000 17:59:23
Target system: System 1
Method last modified by: Florian
Date of last modification: 02.10.2000 17:59:23
Strategy name: B100_100
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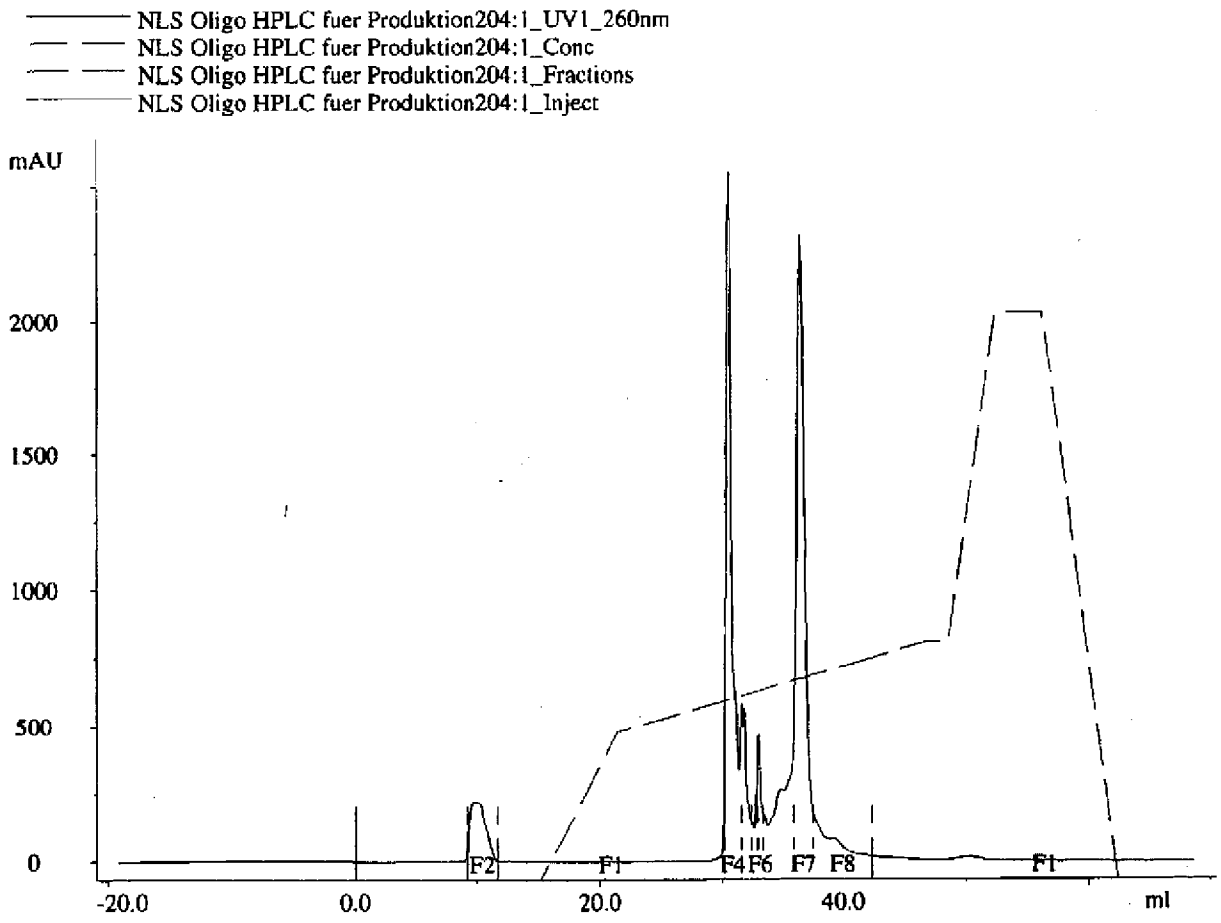
Method file: c:\...\Florian\NLS Oligo HPLC fuer Produktion2



Method Information

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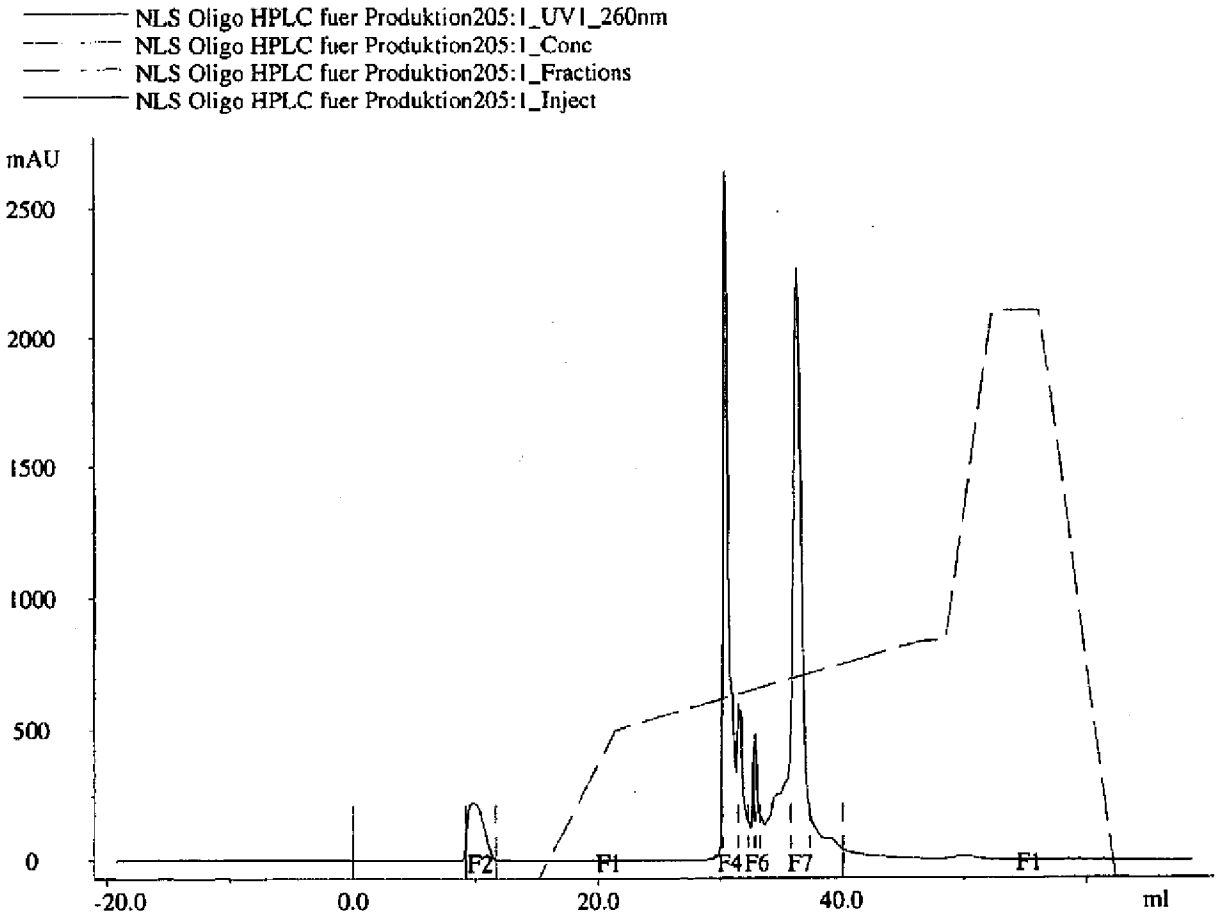
Target system: System 1
Method last modified by: Florian
Date of last modification: 02.10.2000 20:10:03
Strategy name: B100_100
Strategy date: 01.08.2000 14:19:12
Strategy size: 620912 bytes

UNICORN V3.21
Run by: Florian 02.10.2000 20:48:15
Result file: c:\...\Florian\NLS180900\NLS Oligo HPLC fuer Produktion205
Method file: c:\...\Florian\NLS Oligo HPLC fuer Produktion2



Method Information

Method name: NLS Oligo HPLC fuer Produktion205
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Date of creation: 02.10.2000 17:59:23
Target system: System 1
Method last modified by: Florian
Date of last modification: 02.10.2000 20:36:16
Strategy name: B100_100
Strategy date: 01.08.2000 14:19:12
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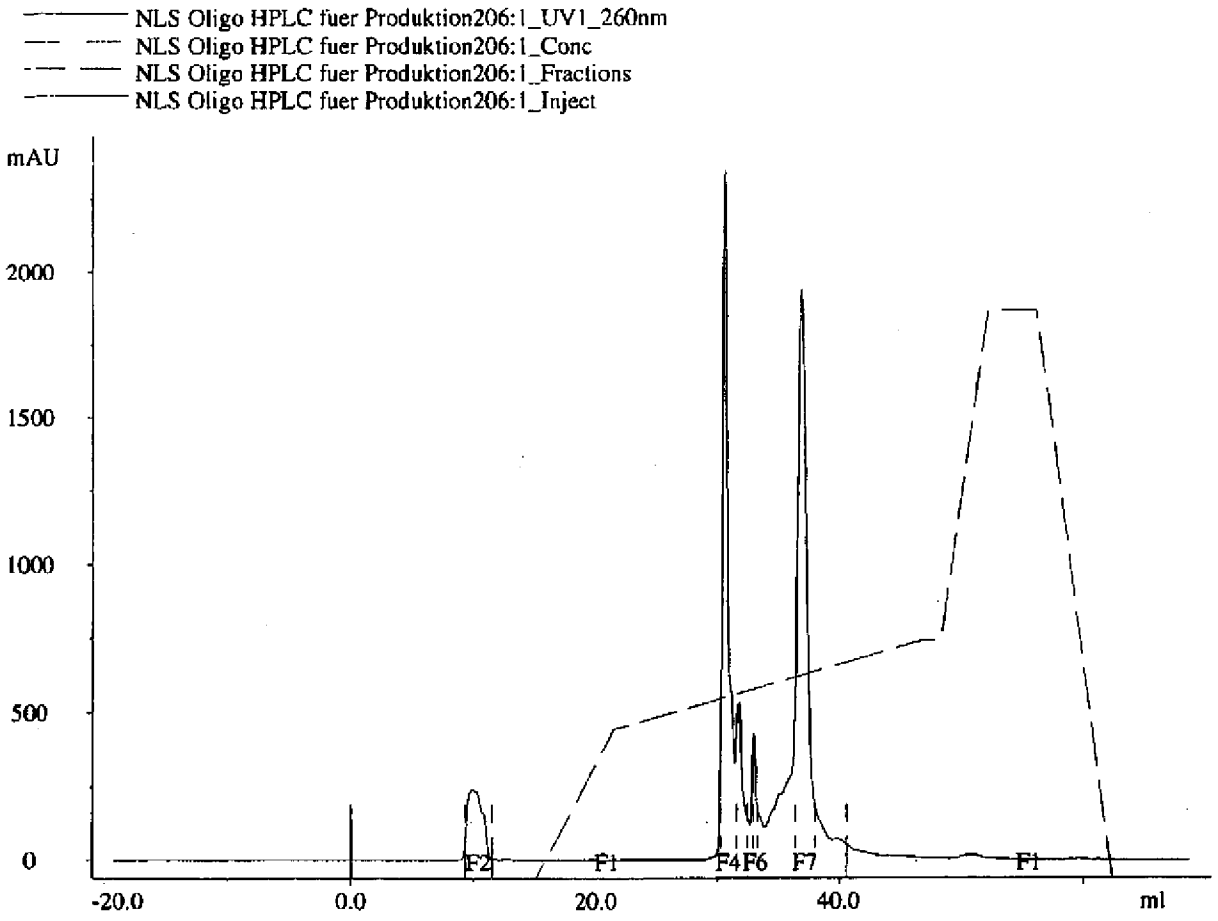
UNICORN V3.21
Run by: Florian 03.10.2000 12:12:17
Result file: c:\...\Florian\NLS180900\NLS Oligo HPLC fuer Produktion206
Method file: c:\...\Florian\NLS Oligo HPLC fuer Produktion2



Method Information

Method name: NLS Oligo HPLC fuer Produktion206
Method created by: Florian
Date of creation: 02.10.2000 17:59:23
Target system: System 1
Method last modified by: Florian
Date of last modification: 02.10.2000 21:13:32
Strategy name: B100_100
Strategy date: 01.08.2000 14:19:12
Strategy size: 620912 bytes

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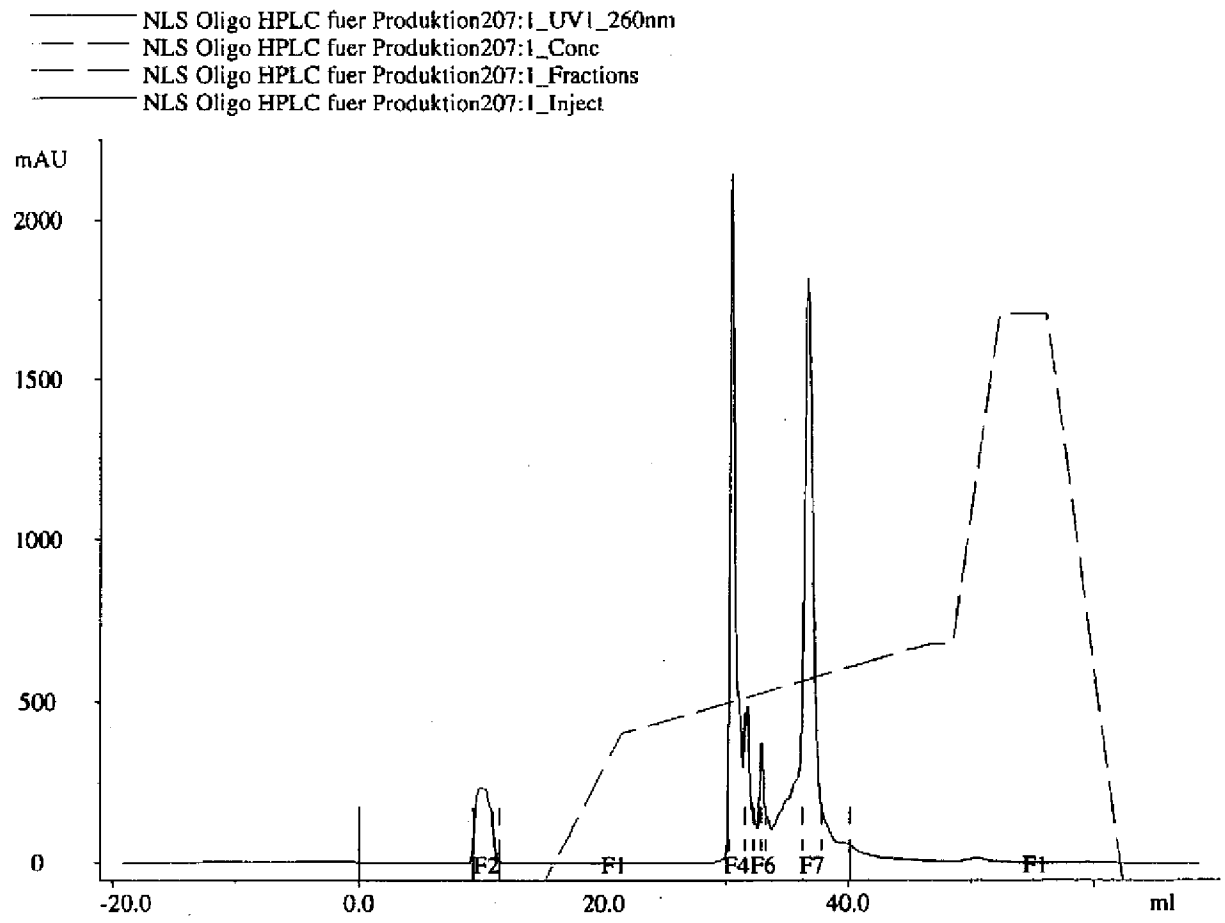
Method file: c:\...\Florian\NLS Oligo HPLC fuer Produktion2



Method Information

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Date of creation: 02.10.2000 17:59:23
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Method last modified by: Florian
Date of last modification: 02.10.2000 21:13:32
Strategy name: B100_100
Strategy date: 01.08.2000 14:19:12
Strategy size: 620912 bytes

UNICORN V3.21
Run by: Florian 03.10.2000 13:27:13
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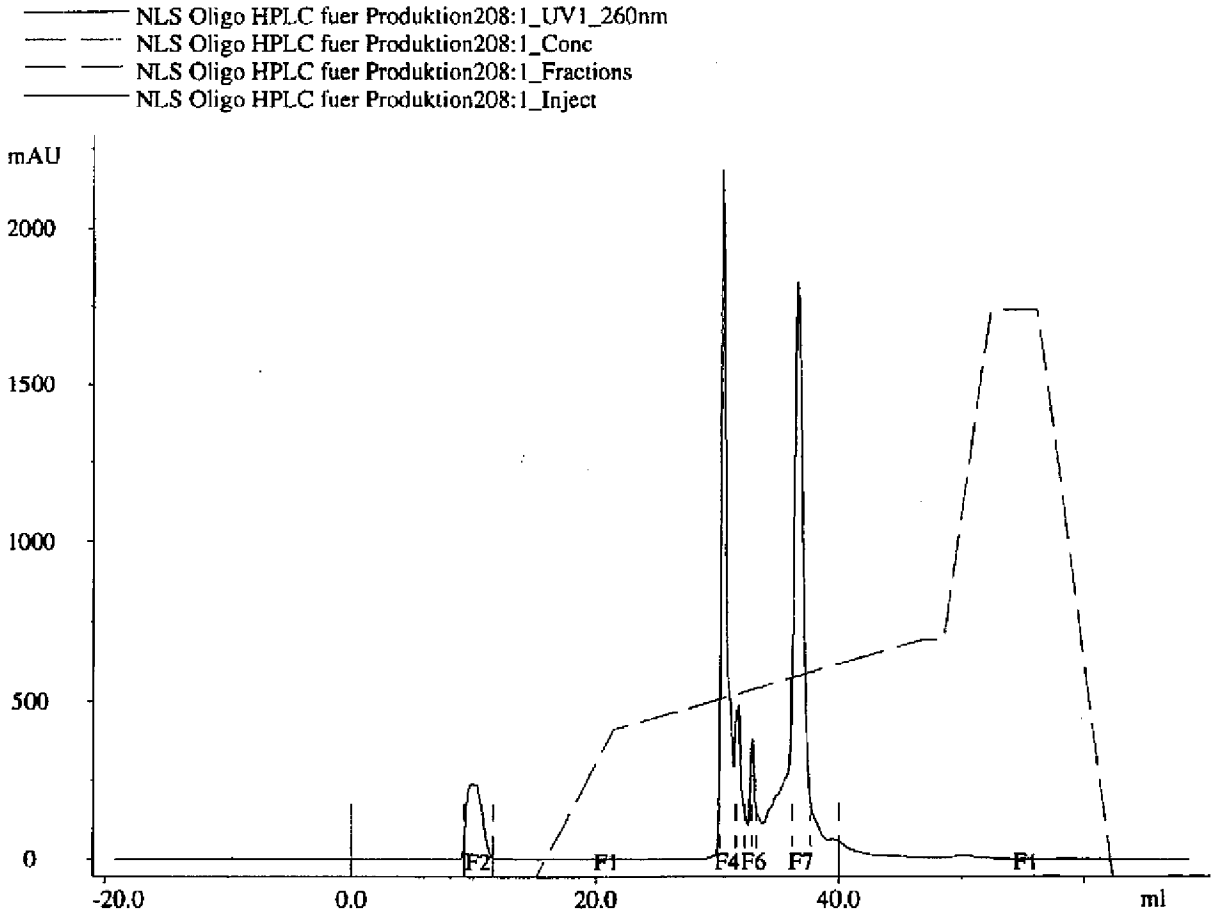
Method file: c:\...\Florian\NLS Oligo HPLC fuer Produktion2



Method Information

Method name: NLS Oligo HPLC fuer Produktion208
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Method last modified by: Florian
Date of last modification: 02.10.2000 21:13:32
Strategy name: B100_100
Strategy date: 01.08.2000 14:19:12
Strategy size: 620912 bytes

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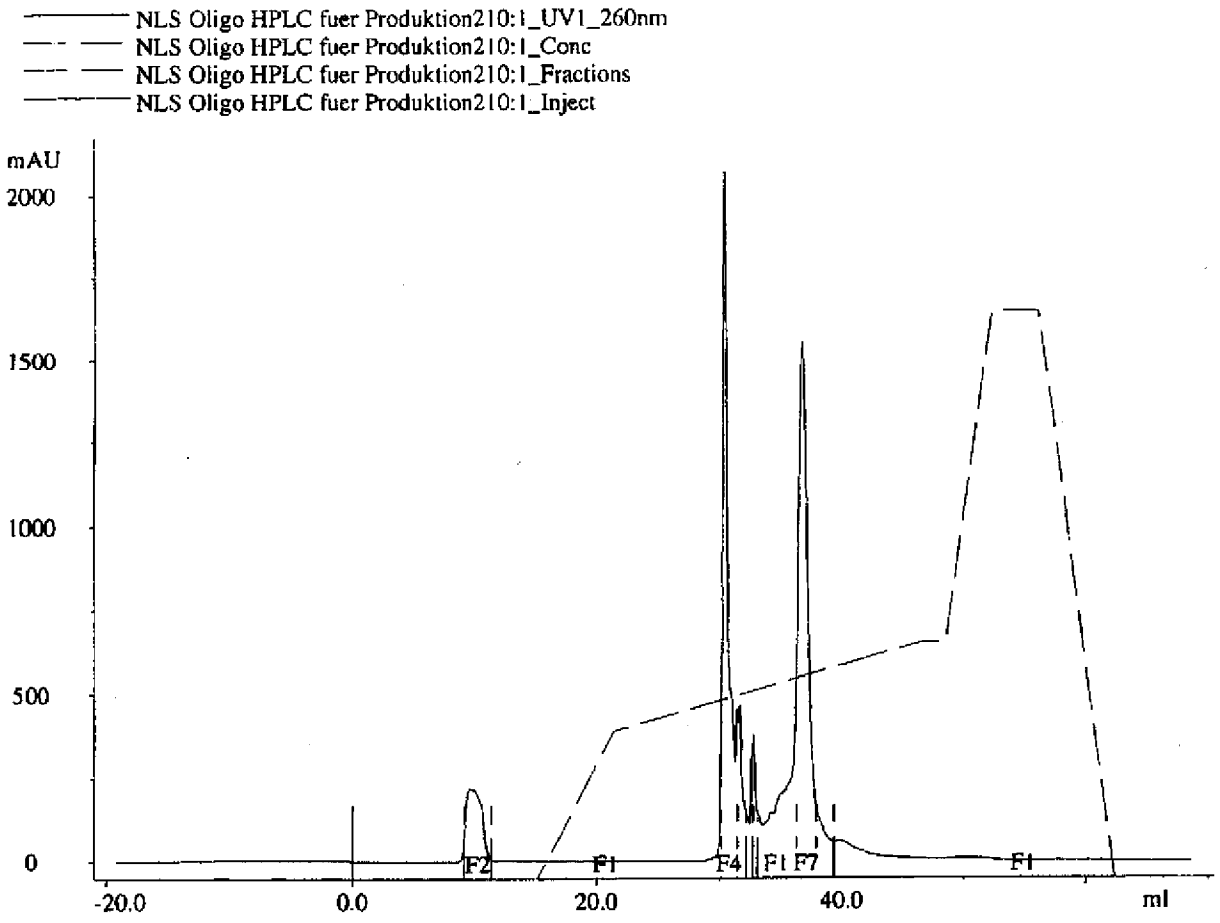
Method file: c:\...\Florian\NLS Oligo HPLC fuer Produktion2



Method Information

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Strategy name: B100_100
Strategy date: 01.08.2000 14:19:12
Strategy size: 620912 bytes
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UNICORN V3.21
Run by: Florian 03.10.2000 16:48:37
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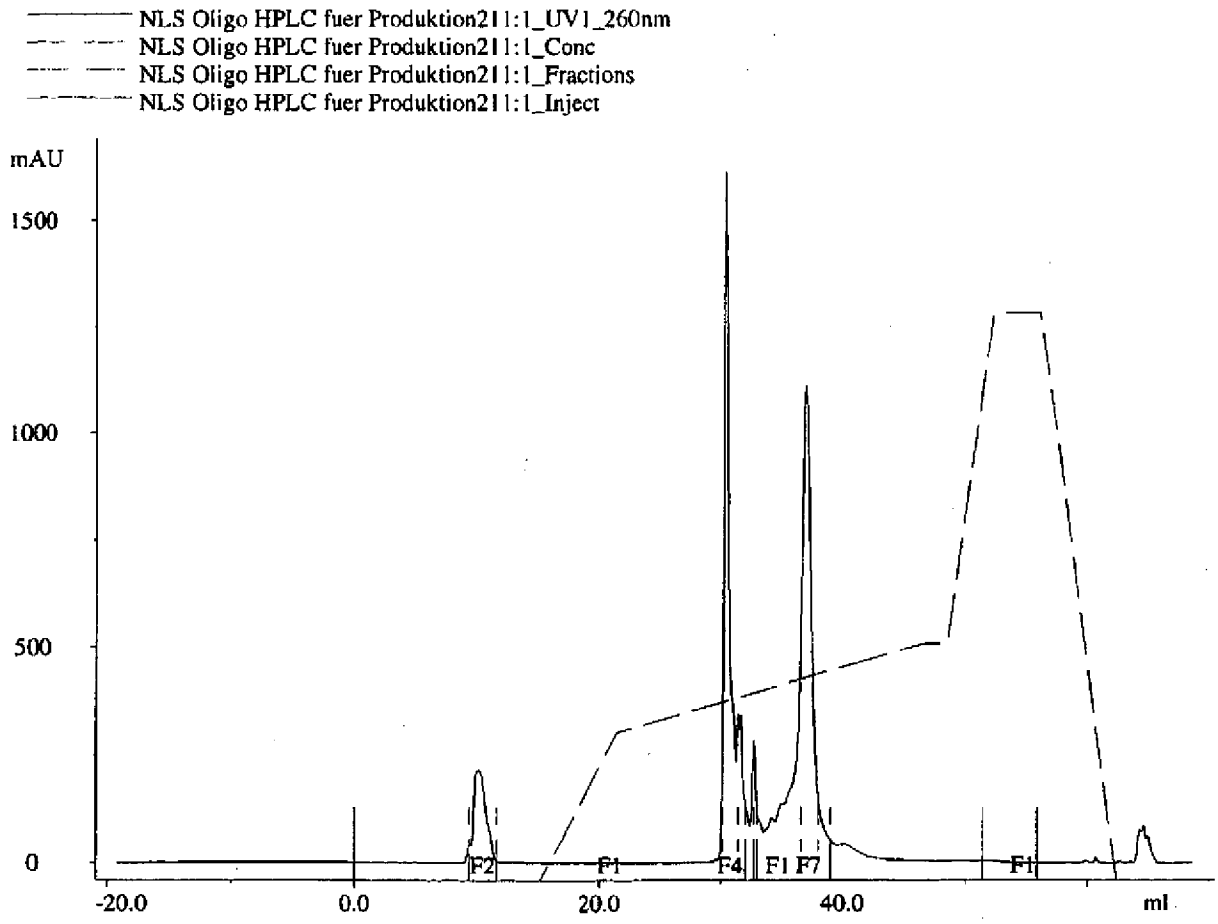
Method file: c:\...\Florian\NLS Oligo HPLC fuer Produktion2



Method Information

Method name: NLS Oligo HPLC fuer Produktion211
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Date of creation: 02.10.2000 17:59:23
Target system: System 1
Method last modified by: Florian
Date of last modification: 02.10.2000 21:13:32
Strategy name: B100_100
Strategy date: 01.08.2000 14:19:12
Strategy size: 620912 bytes

EXHIBIT C

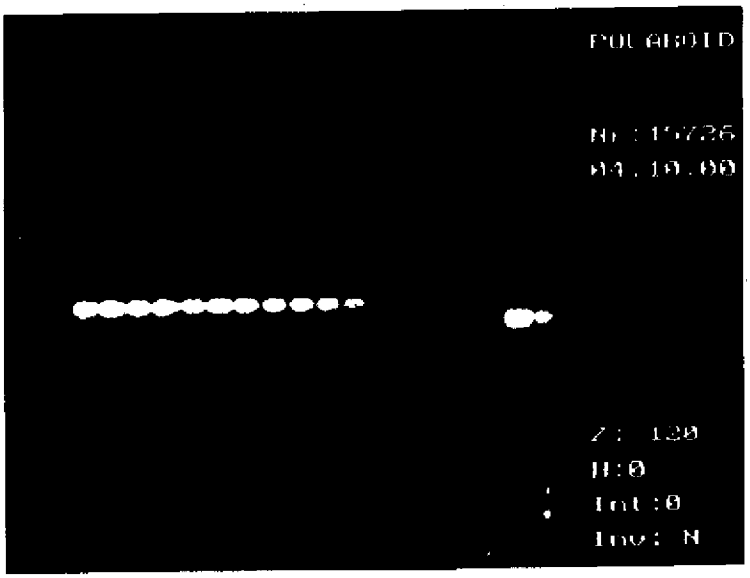
4.11.00

NLS-MDL66CANH nach HPLC +TAT
je 1 µl

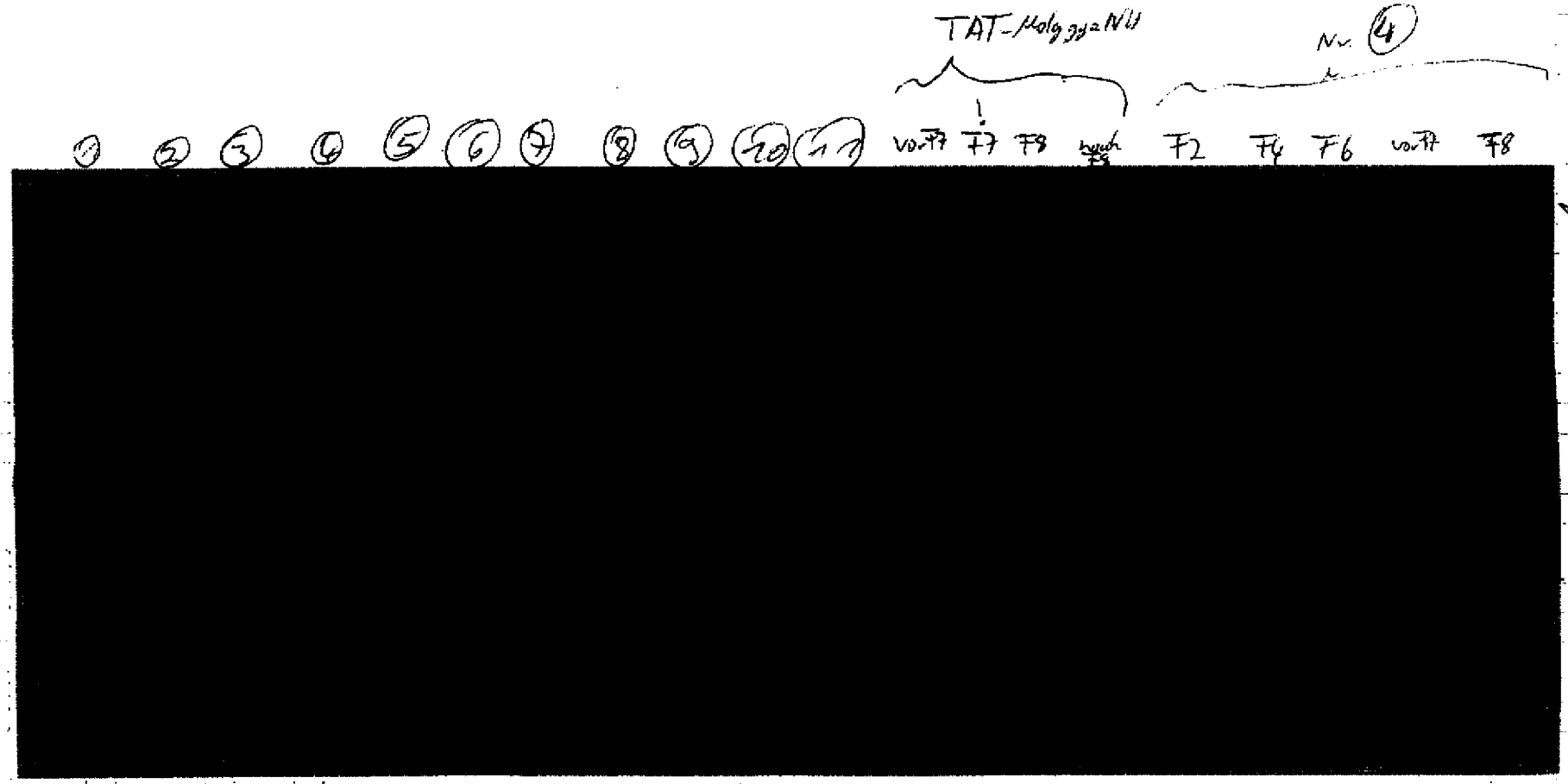
F7 ⑦–⑪ in je 250 µl

↓ TAT HPLC { vor F7 50
F7! 150 µl
F8 50
nach F8 50

	von HPLC-Lauf Nr.	
F2	④	50 µl
F4	④	50 µl
F6	④	50
F vor 7	④	50
F8	④	50



NLSTATHPLCFraktionen



Fortsetzung von S. 82

EXHIBIT D

Chargennummer: 251-00 Produktname: MOL-HBsAGSAY1xNLS-M

2. Ligation (SOP-P-10 Version:_____)

Berechnung der Menge an Oligos: $m_{Oligo\ 1}$ [µg]= 1370 — 20 facher Überschuss
$m_{Oligo\ 2}$ [µg]=137 — 2 facher Überschuss

	Nummer	Konzentration	Volumen [µl]	
Volumen Restriktionsverdau	----	----	51750	
Wasser (frisch entnommen)	----	----	1986	✓
Oligo 1 MOL-D	13611	5 µg/µl	274	✓
Oligo 2 MOL-6661A-NLS	124	1,3 µg/µl	105	✓
ATP	L87134	100mM	565	✓
Puffer G+	NL20	10-fach	471	✓
Eco 31I	69161-63	50 U/µl	1242	✓
T4-DNA-Ligase	35114	5 U/µl	62	✓
Gesamtvolumen		DNA:0,55 µg/µl	46455	
Gefäß		---	150ml	
Datum/Unterschrift	21.12.00 M. Rothe			

Erneute Zugabe von Eco 31I für 2h. 500µl ✓ 69160 M. Rothe 22.12.00 14:18 – 16:11

15- 20 h bei 37°C

Zeit hineingestellt (Datum / Uhrzeit): 21.12.00 / 14:02
Zeit herausgeholt (Datum / Uhrzeit): 22.12.00 / 10:05

3. Probe-T7-Polymeraseverdau (SOP-P-11 Version:_____)

	Nr.	Konzentration	Volumen [µl] Ligation	T7-Verdau
Ansatz	----	----	2 ✓	2 ✓
Wasser	----	----	16,2 ✓	16 ✓
Puffer G+	NL20	10-fach	1,8 ✓	1,8 ✓
T7-Polymerase	7111	10U/µl	-	0,2 ✓
Gesamtvolumen			20	20
Zeit [h]			2	2
Datum/Unterschrift	22.12.00 M. Rothe			

1% Agarosegel: (je 5 µl auftragen)

Ligation akzeptabel? O Ja O Nein

Probe-T7-Polymeraseverdau positiv ? O Ja O Nein

Weiter mit T7-Polymeraseverdau? (Schritt 4) ☒ Ja O Nein

Datum/Unterschrift
Bemerkungen auf der Rückseite: 22.12.00 M. Rothe

Batch No.: 251-00 Product name: MOK-H8sAG.SAY1xNLS-M

2. Ligation (SPO-P-10 Version:_____)

Calculation of oligo primer amounts: $m_{oligo\ 1}$ [µg]= 1370 20 fold excess
$m_{oligo\ 2}$ [µg]= 137 2 fold excess

	Number	Concentration	Volume [µL]	
Volume restriction digest	----	----	51750	✓
Water (freshly removed)	----	----	1986	✓
Oligo 1 MOL-D	136/1	5 µg/µL	274	✓
Oligo 2 MOL-GGGA-NLS	124	1,3 µg/µL	105	✓
ATP	487/3-4	100mM	505	✓
Buffer G+	NL20	10-fold	471	✓
Eco 31 I	69/61-63	U/µL	1242	✓
T4 DNA ligase	35/14	U/µL	62	✓
Total volume		DNA: 0,55 µg/µL	56455	
Reaction tube		----	150 mL	
Date / Signature 12/21/00				

further addition of Eco31I for 2 hours.
500µl 69/60
12/22/00 2:18 pm – 4:11 pm

15 - 20 hours at 37°C

Start of Incubation (date / time): 12/21/00 2:02 pm
End of Incubation (date / time): 12/22/00 10:08 am

3. Test restriction digest of T7-polymerase (SPO-P-11 Version:_____)

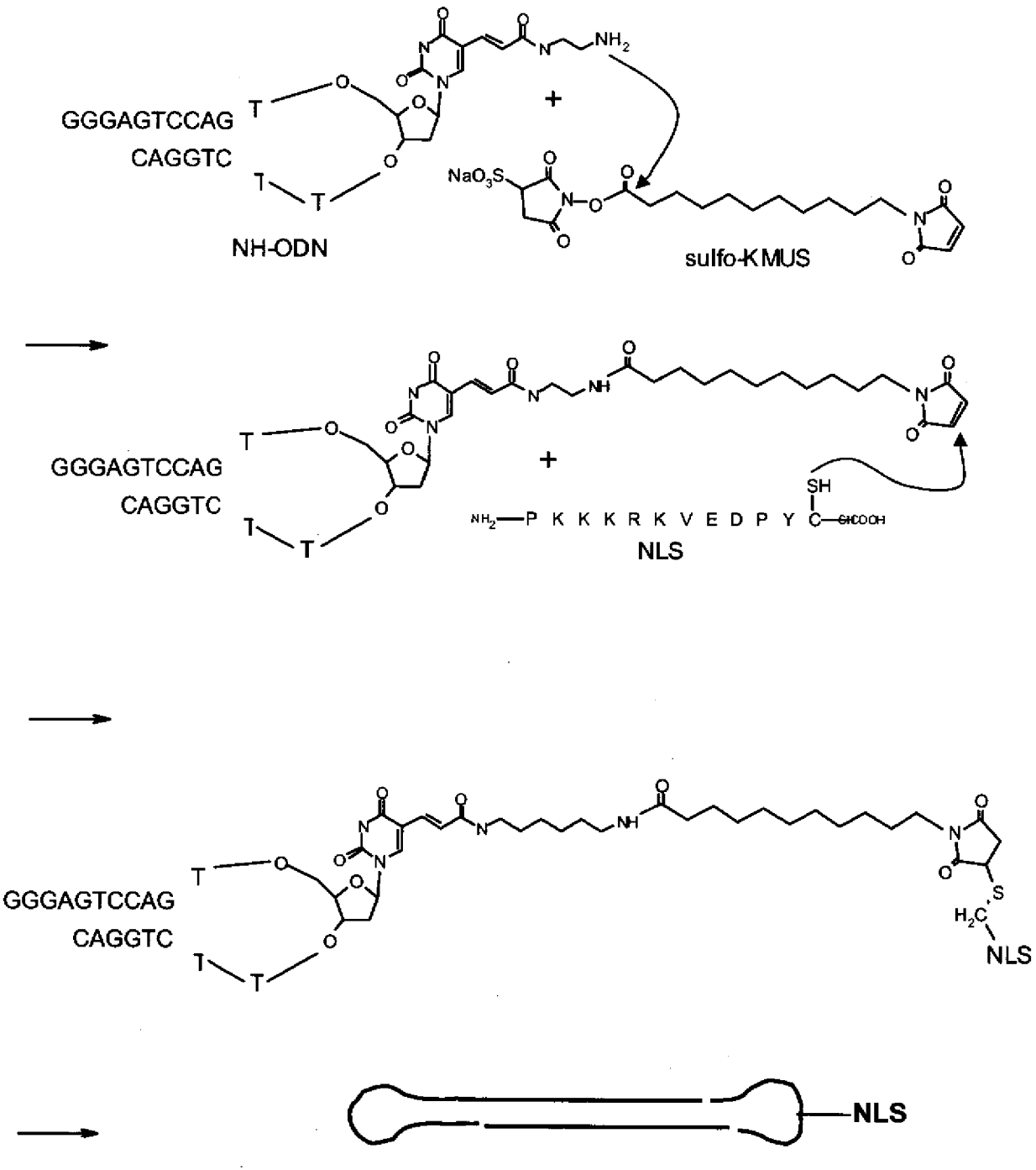
	Number	Concentration	Volume [µL]	
			Ligation	T7 restriction
Reaction batch	----	----	2	2
Water	----	----	16.2	16
Buffer G+	NL20	10-fold	1.8	1.8
T7-Polymerase	71/1	10U/µL	-	0.2
Total volume			20	20
Time [hours]			2	2
Date / Signature 12/22/00				

1% agarose gel: (5 µL per track)

Ligation acceptable? O yes O no
T7-polymerase digest positive? O yes O no
Proceed with T7-polymerase digest? (Step 4) ☒ yes O no

Date / Signature
Remarks see reverse: 12/22/00

EXHIBIT E


GGGAGTCCAG
CAGGTC
NH-ODN
sulfo-KMUS
NLS
NLS